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MARSSONINA LEAF BLOTCH ON THE APPLE ORCHARD IN BATU, INDONESIA

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ABSTRACT

In Indonesia, apple orchards are decreasing because of leaf blotch disease. Marssonina leaf blotch has not been widely known and the problems are associated with the incidence of the epidemic so they need to be studied. Research aimed to investigate the biological aspect of Marssonina leaf blotch, the resistance of apple varieties and the alternative host of *Marssonina coronaria,* and the interactions between Marssonina leaf blotch and weather factors. The research was conducted in laboratory, greenhouse and on apple orchard respectively. There are three results in this research. First, fruit bodies (apothecia) are not produced by fungus, but only conidia (imperfect form) are. Second, based on bioassay, pathogen survives only on the apple. Finally, there are three formula can be used to predict the leaf blotch disease, such as:1) $Y=-37.91+19.98X_6$ (population of spores in the air),2) $Y = -438.13 + 25.71X_1$ (temperature) $-3.05X_2$ (humidity) $+ 41.07X_3$ (wind speed) $-2.07X_4$ (sunlight) $+ 19.25X_5$ (rainfall), and 3) $Y = -43.86-1.61\ 22.60\ X_5$ (rainfall) $+ X_6$ (population of spores).

Keywords: apple; forecast; leaf blotch disease; *Marssonina coronaria*

INTRODUCTION

The centers of apple production in Indonesia are located in Batu and Malang. Rome Beauty and Manalagi are the two major varieties in both areas. Recently, the planting area is decreasing because of leaf blotch disease that can produce yield loss up to a hundred percent. Leaf blotch caused by *Marssonina coronaria* was reported in Italy (Tamietti and Matta, 2003; EPPO, 2013), India (Sharma *et al.*, 2004) and South Korea (Lee *et al*., 2011).

In Indonesia, Marssonina leaf blotch has not been widely known so that the problems are associated with the incidence of the epidemic and it needs to be studied. Biological, ecological aspects of pathogen and interaction of weather factors on the disease incidence were studied in this research to predict the fluctuation of Marssonina leaf blotch.

MATERIALS AND METHODS

There were three activities in this research: 1) biological studies of Marssonina leaf blotch, 2) studies of the resistance of apple varieties and the alternative host of *M. coronaria,* and 3) interactions between Marssonina leaf blotch and weather factors. The research was conducted from 2011 to 2013 to cover all research activities.

Biological Studies of Marssonina Leaf Blotch

To observe biological aspect of Marssonina leaf blotch, there were two experiments conducted in the laboratory. The objectives were to determine the influence of the medium, and to observe the influence of weather factors for the growth of pathogenic fungi.

The first experiment was carried out as follows: 1) Preparation of eight types of artificial media i.e.: water agar (WA), potato saccharose agar (PSA), potato sucrose agar (PScA), potato carrot agar (PCA), corn meal agar (CMA), potato dextrose agar (PDA), potato carrot saccharose agar (PCSA) and potato corn meal agar (PCMA). The formula of each of medium was described by Tuite (1969); 2) All artificial media were prepared in a 250 ml glass bottle (Erlenmeyer, Pyrex USA), then sterilized in an autoclave at 121^{o}C for 15 minutes with the power pressure of 0.7 kg cm^{-2} (Sastrahidayat and Djauhari, 2012); 3) Once ster-

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ilized in the autoclave, the material of each medium was then poured into a Petri dish (90 mm Pyrex glass cover), each type of medium consisted of five petri dishes as replication in treatment; 4) Then in the middle of each petri dish was inoculated one pure culture disc (Ø 5 mm) of *M. coronaria* which taken from on PDA; All treatments were done under sterile conditions and then each inoculated petri dish was incubated at 25^{o}C; and 5) The observations of fungus was done every day by measuring the development of colonies, and the appearance of mycelial colonization.

In a second experiment, the effect of temperature on the germination of spores, germ tube length and colony growth of pathogenic test was done. To determine the effect of temperature on the growth of *Marssonina coronaria, a test* was done by inoculating one pure cultures disc (Ø 5 mm) of pathogens on to the middle of PSA medium in petri dishes (90 mm). *Marssonina coronaria* culture was diluted with sterile distilled water using a test tube shaker at 2800 rpm for 30 seconds, then 10 ml was taken by using a pipette and put into a 10 ml sample bottle screw top clear glass. Thereafter, the Petri dishes and bottle vials was incubated at 10, 15, 20, 25 and 30°C.The growth of colony was observed daily by measuring the diameter of colonies. While the germination percentage of spores and the tube length were observed under the light microscope (400 x) at 6, 12 and 24 hours after inserted into the incubator.

The effect of light on percentage, tube length of germinations, and the growth of colonies tests were also conducted in the same procedures as previous. For lighting effect test, 1.5 x 1.5 x 1.5 m of plywood box as growth chamber was exposed by a 10 watt fluorescent lamp and near ultraviolet (NUV). The treatment of light was composed by 24-hours of light, 24-hours of dark, 12-hours of light then followed by 12-hours of dark, 24-hour near ultraviolet light, 12-hours of ultraviolet light and 12-hours of darkness. The growth of fungal colony was observed daily by measuring the diameter of colonies on the petri dish culture.

Both experiments were designed by a fully randomized design (FRD), which was repeated five times. To see the differences between the treatments, then data was analyzed by analysis of variance at 5% of α (Steel and Torrie, 1980) and the average was compared using Duncan Multiple Range Test (DMRT). SPSS-16 software were used for processing the data.

Resistance of Apple Varieties and Alternative Host of *M. coronaria*

This activity was conducted in the green house. Activity was aimed to determine the resistance of apple varieties and the possibility of alternative hosts for *M. coronaria*. The apples were consisted of wild apple, Rome Beauty, Manalagi and Princes Noble varieties for resistance test. Pear, rose, mulberry, and strawberry were used as compared hosts.

All plant samples were planted in plastic pots (ten kilograms soil per pot) and placed in the green house of the Faculty of Agriculture, University of Brawijaya. On the leave of each plant sample was then sprayed (using a hand sprayer, one liter of volume) by *M. coronaria* spores which was taken from pure cultures in PSA with a concentration of 9 x 10^6 spores ml^{-1} sterile distilled water. After that, each plant sample was covered with transparent perforated plastic for two to three days in order to obtain high humidity which useful for the pathogen infection. To determine the effect of infection on Manalagi and Rome Beauty varieties, were used as objects by spraying (with hand sprayer), wounding (ooze needle), slicing (knife) of fungal inoculum with the same concentration. Inoculated fruits were then incubated in a plastic box with high humidity (ca. 100%).

The number of spots both on the leaves and fruits was counted. Data were analyzed by analysis of variance at 5% of α. For the responses of host plants, positive (+) and negative (-) symbols were used for infected and uninfected responses respectively.

Interactions between Marssonina Leaf Blotch and Weather Factors

Disease incident in the field was caused by the weather factors and biotic interaction. Path analyses were used to clarify the interaction between disease intensity, weather factors (such as temperature, relative humidity, rainfall, light period, wind velocity) and number of spores in the air. The experiment was conducted on apple orchard in Batu by choosing twenty plant samples randomly. To know the infection on the leaves, there was no fungicide application after the shoot. This condition was made to stimulate the disease incident as natural as possible. Soon after defoliation condition, all weather factors were recorded automatically in weather station with closed to the experimental location. A number of

spore in the air was caught by three rotor sampler in the three locations on the field. Monitoring was done every day started from defoliation to harvest. Disease intensity is counted by scoring system (Sastrahidayat, 2013a).

RESULTS AND DISCUSSION

Biological Studies of *Marssonina coronaria*

Morphological Characteristic of Marssonina coronaria

A fungal fruiting bodies (acervulus) produced conidia masses (Figure 1). Conidia formed on the pedicel in the acervulus, with the pointed tip position conidia attached to the pedicel, while the blunt end facing up. Conidia, obovoid, hyaline, a septate, ranged between 14.8-21 x 3.5-5.3 µm in size. This characteristic is similar to *M. coronaria* that described by Lee *et al.* (2011), however there are differences in the size of the conidia. When conidia were germinated in sterile water medium on a glass slide convex, then within six hours, it formed germ tubes. It ranged between 12 to 24 hours after the time of inoculation, tube length reached about 15-24 µm. Each conidium is only one germ tube, formed by percurrent, lateral, and semi axial (Figure 1). According to Sharma *et al.* (2009), 20^{o}C of temperature and 100% of relative humidity, conidia of *M. coronaria* can germinate optimally. These observations indicated that leaf blotch on apple was caused by *M. coronaria* (teleomorph: *Diplocarpon mali*). In addition, this result showed the similarity with the results of Tamietti and Matta (2003) on an apple orchard in Italy.

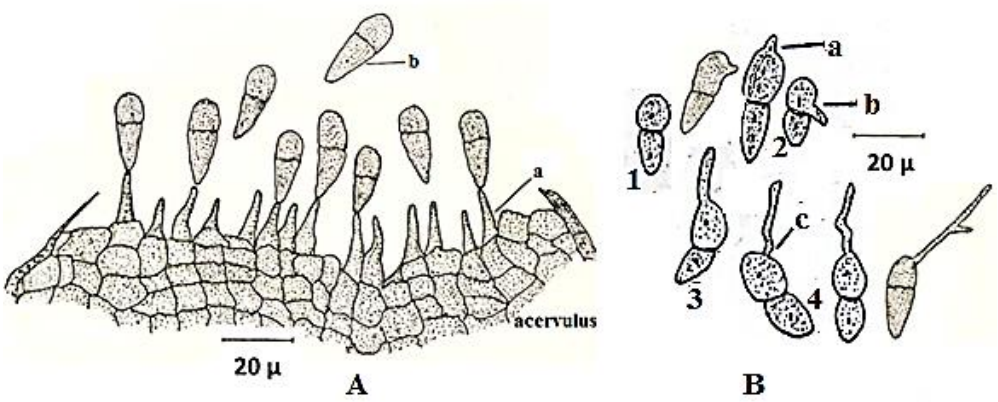

Figure 1. Morphology acervulus and conidia of *Marssonina coronaria*, the cross-section of apple leaves infected (A); and conidia germination process (B), with description: 1, germinated conidia; 2, 3, and 4, germinate after 6, 12, and 24 hours respectively; a, per current; b, semi-axial, c, lateral. Observed by light microscope (400x) (Sharma *et al.*, 2009).

Table 1. The differences of *Marssonina coronaria* growth on eight artificial agar media based on diameter and status of colonies in a week after inoculation

Types of media	Colony diameter (cm)	Description
Water agar (WA)	5.83	Slight
Corn meal agar (CMA)	8.33	Slight
Potato corn meal agar (PCMA)	8.70	Moderate
Potato carrot agar (PCA)	8.78	Moderate
Potato dextrose agar (PDA)	8.88	Moderate
Potato saccharose agar (PSaA)	9.23	Abundant
Potato carrot saccharose agar (PCSA)	9.23	Abundant
Potato sucrose agar (PSuA)	9.24	Abundant

From the observation on the infected parts of the plant either on the leaves, twigs and fruit there were no fruit bodies (apothecia) that observed, which are generally found in the litter on the ground in temperate regions (EPPO, 2013). So it can be assumed that in the tropical regions, these pathogens only survive from season to season in the form of resistant mycelium at litter of apple crop.

The Suitability of M. coronaria on Eight Artificial Media

The fungus had different response to the media. Table 1 showed that on water agar, growth of fungi was very poor. Growth of fungi was moderate on corn meal, potato corn meal, potato carrot and potato dextrose agars; but showed good colonization and abundant with mycelium on potato saccharose, potato carrot saccharose and potato sucrose agar media.

This result showed that the richer sugar media such as PSaA, PCSA and PSuA, the better nutrient for fungi growth, as mentioned by Bilgrami and Verna (1978). Similar results are presented by Zhao *et al.* (2010) show that the potato carrot dextrose broth(PCDB) and potato carrot broth sucrose (PCSB) are suitable for *M. coronaria* (teleomorph: *Diplocarpon mali*) in cases of mycelial growth and production of conidia. Meanwhile the results of Lee *et al.* (2011) shows the highest mycelial growth is observed on mannose and bacto-peptone amended media with colonies 9.0 and 18.5 mm in diameter resulted by using the carbon and nitrogen sources. Pyridoxine and biotin are the best vitamins, while $FeSO_4$ and $FeCl_3$ are the best mineral salt sources for mycelial growth to produce the highest dry mycelial weight.

Effect of Temperature on M. coronaria

In relation to the effect of temperature to the growth of *M. coronaria* showed that the optimum temperature for growth is ca. 25°C. It is similar to Zhao *et al.* (2010) which states that the optimum temperature for fungal growth is around 25°C. This pattern has no difference between observation time from three to six days after inoculation; but after seven days, the optimum temperature range is wider between 20 to 30°C (Table 2). It was suspected because of the limited nutrients with the aging of the growing cultural media. This condition is similar to Steven and Neely (1982) that describe their experiment of *Marssonina juglandis* on walnut, which the optimum temperature of this fungi ranged between 21 to 25°C.

Results of the study on the presence or absence of irradiation on the growth of fungi *M. coronaria*, showed the fungus responses on stimulation irradiation (Table 3).

Table 2. Effect of temperature on the growth of *Marssonina coronaria*

Temperature (°C)	Diameter of fungal colonies on petri dish (cm)				
	3 dai	4 dai	5 dai	6 dai	7 dai
10	0.71*a*	0.99*a*	1.09*a*	1.24*a*	1.37*a*
15	1.47*b*	1.73*b*	1.97*b*	2.23*b*	2.44*b*
20	1.99*c*	2.33*c*	2.65*c*	2.84*c*	2.96*c*
25	2.19*d*	2.59*d*	2.93*d*	3.07*d*	3.07*c*
30	1.97*c*	2.32*c*	2.64*c*	2.97*c*	3.05*c*

Remarks: DAI is days after inoculation; the numbers are accompanied by the same letter within a column are not significantly different means (in test 5%) by the DMRT

Table 3. Effect of light on the growth of *Marssonina coronaria*

Light treatment	Diameter of fungal colonies in petri dish(cm)				
	3 dai	4 dai	5 dai	6 dai	7 dai
NUV 24 h	4.08	6.04	6.87	8.45	8.95
NUV 24 h/dark 12 h	4.86	6.88	8.22	8.81	9.00
Light 24 h	1.78	4.96	7.19	8.99	9.16
Dark 24 h	1.66	4.36	5.18	7.81	8.15
Dark 12 h/light 12 h	1.80	5.88	8.02	9.09	9.30

Remarks: DAI = days after inoculation, NUV = near ultra violet

Table 4. Effect of temperature on the conidial germination and length of germ tube of *Marssonina coronaria*

Temperature (^{0}C)	Hours after Inoculation	Conidial germination (%)	Length of germ tube (µm)
10	6	3.62	14.81
	12	4.75	15.57
	24	5.48	19.77
15	6	5.65	15.37
	12	8.13	16.41
	24	12.82	20.84
20	6	8.61	25.27
	12	12.37	31.64
	24	14.60	43.66
25	6	6.73	17.79
	12	8.83	20.96
	24	13.93	24.10
35	6	3.08	11.98
	12	5.62	18.95
	24	5.46	19.08

Table 5. Total spot and spot area on apple leaves caused by *Marssonina coronaria*

Apple varieties	The number of spots and spot areas, 21 days after inoculation	
	∑ leaf blotch	Leaf blotch diameter (mm)
Wild	0.84 a	2.77 a
Rome Beauty	1.12 b	3.93 b
Manalagi	1.06 b	3.59 b
Prince Noble	0.76 a	1.23 a

Remarks: The numbers are accompanied by the same letter within a column are not significantly different means (at α=5%) by the DMRT

Table 3 shows that near ultra violet (NUV) stimulated early growth of fungi, both given for 12 hours followed by 12 hours and then continued for 24 hours without the dark. This result gave an impression that ultra violet even in a little dose was very important for fungi growth; unfortunately there was no indication has been obtained on how many hours or minutes the lowest of ultra violet exposure. As also shown in Table 3, the fungal growth was stimulated by light Twi lighting (TL), will delay the fungi. Harada *et al.* (1974) states that light intensity lower than 500 lux for 12 hours exposure stimulated *M. coronaria* development.

The result showed that germination and growth of germ tube were influenced by the temperature. As also shown in Table 2, the temperature ranged between 20 to 25°C indicated an optimum temperature especially for the growth of germ tube. This tendency also occurred for conidial germination (Table 4).

Resistance of Apple Varieties and Alternative Hosts of *Marssonina coronaria*

The results of artificial inoculation of pathogens to apple leaf of wild type, Rome Beauty, Manalagi and Princes Noble; showed a different response (Table 5). Table 5 showed after 21 days of inoculation, the number of spot and its area on the Rome Beauty and Manalagi varieties were broader than the Princes Noble and wild type. This resistance difference is suspected related to physical endurance (morphology) of the apple leaf. Based on the results of microscopic observation, showed that the Princes Noble and wild types had trichomes ca. 304 and 382 per mm of leaf area respectively; while in Rome Beauty and Manalagi had ca. 184 and 192. Agrios (2005) describes the trichomes are some structural defenses that present in the plant even before the pathogen comes in contact with the plant. Such structures include the amount and quality of wax and cuticle that cover the epidermal cells, the structure of the epidermal cell walls, the size, location, and shapes of stomata and lenticels, and

the presence of tissues made of thick-walled cells that hinder the advance of the pathogen on the plant. Regarding the possibility of different chemical compounds in each type of apple, a further investigation was not conducted; but it was suspected that sugar contain compounds were higher in Rome Beauty and Manalagi and helped the sensitivity of these plants to pathogens based on bioassays.

If the increasing of total spotting and spotting areas were analyzed statistically, it appears the logistics relationship from one to two weeks after inoculation; with the value of the correlation coefficient (r), 0.79 and 0.94 respectively. After three weeks, the value of the correlation coefficient declined sharply (r = 0.33). The decreasing is related to the weather conditions in the experimental environment, particularly low humidity (below 75%) and an increase in air temperature (above 25^{o}C). Li *et al.* (2011) suggests that there is a relationship between humidity and air temperature on the incidence of the apple leaf blotch disease.

Conidia inoculation on fruits by three methods (sprayed, sliced and pricked) to Manalagi and Rome Beauty varieties showed interaction between the treatments (Table 6).

Table 6 shows that the method of inoculation by spraying conidia on fruit was ineffective to *M. coronaria* infection, both in Manalagi and Rome Beauty varieties. In contrast to the method of wounding, either sliced or prickled showed effective results for the pathogen infection in both varieties. However, if the two varieties were compared on their susceptibility to infection and seen from the rotten fruit on day 14, Manalagi was more sensitive than Rome Beauty. This sensitivity seems to be related to the structure of the physical endurance of fruit, especially the thickness of the skin. In relation to the thickness of apple skin, Kusumo (1986) reports that Rome Beauty variety has thicker cuticles than Manalagi.

Experiments by inoculating conidia of *M. coronaria* on some leaves of other plants (pear, rose, strawberry and Mulberry) showed that the symptoms did not appear on the test plants until several weeks after inoculation; while on the apple, the symptoms occurred only in one week. Those facts showed that the possibility of other hosts besides apples for *M. coronaria* was uncertain, so the follow up research was needed especially in the family Rosaceae.

Interactions between Marssonina Leaf Blotch and Their Weather Factors

Symptomatology

Based on the field observations, symptoms were started from the small of spots on the leaves with irregular shape, somewhat precipitate, then they fused and produced the dying leaves and the dark brown spots. As a result of severe attack, all leaves would fall and then plant became bald, die, and failed to produce fruits. Symptoms are described in Figure 2.

Table 6. Rate of fruit rot on apple inoculated by *Marssonina coronaria*

Variety/method of inoculation	Rate of fruit rot				
	6 dai	8 dai	10 dai	12 dai	14 dai
Manalagi:					
Sprayed	0.03*ab*	0.03*ab*	0.03*ab*	0.03*ab*	0.03*ab*
Slice	3.13*c*	4.58*c*	6.00*c*	7.25*d*	8.88*c*
Pricked	2.70*bc*	3.23*b*	4.20*bc*	5.30*c*	6.93*b*
Rome Beauty:					
Sprayed	0.00*a*	0.00*a*	0.00*a*	0.00*a*	0.00*a*
Slice	2.33*b*	3.23*b*	3.78*b*	3.92*b*	5.25*b*
Pricked	2.30*b*	3.00*b*	3.98*c*	4.03*b*	5.63*bc*

Remarks: DAI is day after inoculation; the figures are accompanied by the same letter within a column are not significantly different means (at α=5%) by the DMRT

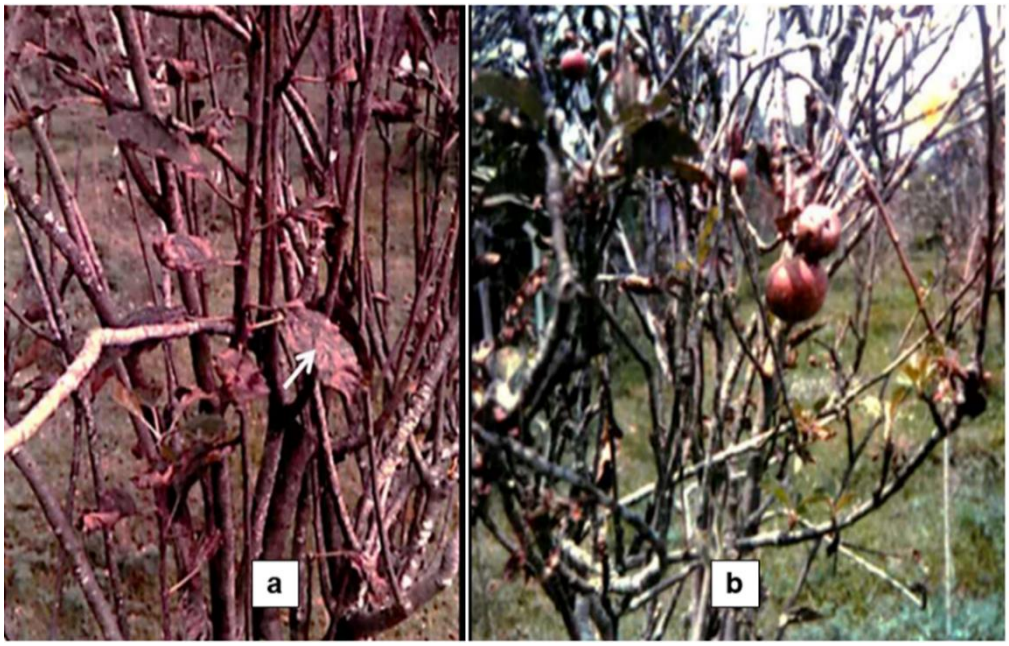

Figure 2. Marssonina leaf blotch a) symptom on apple's leaves and b) the impact on fruit production

Epidemiological Studies

The results of statistical analysis of the data on the incidence of leaf blotch disease of apples in the field showed that there was a close relationship between weather factors, the population of conidia in the air, and the intensity of disease (Table 7). The weather factors that consisted of air temperature (X_1), humidity (X_2), wind speed (X_3), duration of solar radiation (X_4), rainfall (X_5), along with a population of conidia in the air, showed that there was a relationship between all factors to the disease percentage (value of r 0.93) (Table 7). If the population of conidia were excluded from these interactions, the relationship would tend to decline with r value, 0.88. Based on r value, the interaction between all tested weather factors influence the intensity of disease. The population of conidia in the air affected the intensity of disease with the r value, 0.80. In the case of rain fall, it had no effects to the intensity of disease, if it was a single factor (r value: 0.61). If the rain fall was combined with the population of conidia, they affected the intensity of disease with r value, 0.81.

There were positive linear relationship between rainfall and population of conidia in the air to the intensity of disease. Li *et al.* (2011) shows that there is a relationship between temperature and humidity to the incidence of disease, however based on bioassay, there is no relationship between them.

The incident in epidemiological studies of plant diseases is not surprising, given the complex interactions such weather factors for each region, where Li *et al*. (2011) studied in China (subtropical area), whereas the results of this study are events in Batu, Indonesia (tropical area). It can be explained in more detail when the correlation coefficients were analyzed in an integrated manner using path analysis (Sastrahidayat, 2013b). Then it appears that the slightest role partially weather factors, will determine the dynamics of the relationship of other factors in the field.

Based on high r value and the significance analysis of variance (at α=5%), there are some approach can be used as a tool to predict the leaf blotch disease on apples. The equation can be used as a forecast tool, based on daily weather observation data on the incidence of the disease three days ahead. So there is enough time to carry out preventive measures with fungicides, if there were indications of the emergence of a disease outbreak, for three to five days. According to Kumar and Sharma (2014) dithiocarbamates (mancozeb and metiram) are found as the best protectants for Marssonina blotch of apple in the semi-controlled as well as under field conditions.

Table 7. The relationship between weather factors and Marssonina leaf blotch

No.	Regression	R	Expl.
1	$Y = -43.86-1.61 X_5+ 22.60 X_6$	0.81	Significant
4	$X_6= 12.11-1.42 X_3$	0.71	Significant
5	$Y = -37.91+19.98 X_6$	0.80	Significant
6	$Y = 21.51+7.59 X_5$	0.61	Significant
7	$Y = -186.07+5.66X_1 -1.83X_2 +18.53X_3 + 0.39X_4+8.91X_5 +23.20X_6$	0.93	Significant
8	$Y =-438.13+25.71X_1-3.05X_2+41.07X_3-2.07X_4+ 19.25X_5$	0.88	Significant
9	$Y = -43.86-1.61 X_5+22.60 X_6$	0.81	Significant

Remarks: X_1 = air temperature (°C); X_2 = air humidity (%); X_3 = wind speed (km h^{-1}); X_4 = sunlight duration (hour day^{-1}); X_5 = rainfall (ml day^{-1}), X_6 = the population of conidia (per view of field); Y= the intensity of the disease (%)

CONCLUSION

The study did not reveal any fruit bodies (apothecia) as the result of the fungal mating (Perfect form), so that infectious pathogens on plants (especially the leaves) was only through the inoculum in the form of conidia (imperfect form).

From season to season (for two years), these pathogens survived only on the apple crop, because there was no infection on several plants (roses, strawberry) based on bioassays.

The incidence of the disease and the high level of pathogens on apple were strongly influenced by the dynamics of the weather condition. There are three formulas that can be used to predict the leaf blotch disease on the apple, such as:

1) $Y = -37.91 + 19.98 X_6$ (population of spores),
2) $Y = -438.13 + 25.71 X_1$ (temperature) $-3.05 X_2$ (humidity) $+ 41.07 X_3$(wind speed) $-2.07 X_4$ (sunlight) $+ 19.25 X_5$ (rainfall),
3) $Y = -43.86-1.61\ 22.60\ X_5$(rainfall) $+ X_6$ (population of spores)

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REFERENCES

Agrios, G.N. 2005. Plant patology, fifth edition. Elsevier Academic Press. Burlington. p. 952.

Bilgrami, K.S. and R.N. Verma. 1978. Physiology of fungi. New Delhi: Vikas Publishing House. p. 507.

EPPO. 2013. *Diplocarpon mali* (anamorph: *Marssonina coronaria*): Marssonina blotch of apple. European and Mediterranean Plant Protection Organization. Paris. http://www.eppo.int/QUARANTINE/Alert_List/fungi/Diplocarpon_mali.htm. Accessed on December 16, 2015.

Harada, Y., K. Sawamura and K. Konno. 1974. *Diplocarpon mali* sp. nov., the perfect state of apple blotch fungus *Marssonina coronaria*. Annals of the Phytopathological Society of Japan 40 (5): 412-418. doi: 10.3186/jjphytopath.40.412

Kumar, A. and J.N. Sharma. 2014. Monitored control of Marssonina blotch of apple caused by *Marssonina coronaria*. Indian Phytopathology 67 (1): 70-76.

Kusumo, S. 1986. Apel (*Malus Sylvestris* Mill) (in Indonesian). Jakarta: Yasaguna. p. 131.

Lee, D.H., C.G. Back, N.K. Win, K.H. Choi, K.M. Kim, I.K. Kang, C. Choi, T.M. Yoon, J.Y. Uhm and H.Y. Jung. 2011. Biological characterization of *Marssonina coronaria* associated with apple blotch disease. Mycobiology 39 (3): 200-205. doi: 10.5941/MYCO.2011.39.3.200

Li, J., L.X. Gou, X.M. Hu, F.P. Ren, J.F. Wei and D.R. An. 2011. Effects of climate factors on the epidemic of apple Marssonina blotch in Shaanxi Province and related prediction models (in Chinese). Ying Yong Sheng Tai Xue Bao 22 (1): 268-272.

Sastrahidayat, I.R. and S. Djauhari. 2012. Phytopathology research techniques (plant pathology) (in Indonesian). Malang: UB Press. p. 174.

Sastrahidayat, I.R. 2013a. Theoretical Epidemiology of plant diseases (in Indonesian). Malang: UB Press. p. 178.

Sastrahidayat, I.R. 2013b. Epidemiology quantitative of plant diseases (in Indonesian). Malang: UB Press. p. 214.

Sharma, N., V.S. Thakur, J. Mohan, S.M.P. Khurana and S. Sharma. 2009. Epidemiology of Marssonina blotch (*Marssonina coronaria*) of apple in India. Indian Phytopathology 62 (3): 348-359.

Sharma, J.N., A. Sharma and P. Sharma. 2004. Out-break of marssonina blotch in warmer climates causing premature leaf fall problem of apple and its management. Acta Horticulturae 662: 405-409. doi: 10.1766 0/ActaHortic.2004.662.61

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach, 2nd edition. New York: McGraw-Hill. p. 633.

Steven, C. and D. Neely. 1982. Penetration and infection of leaves of black walnut by *Marssonina juglandis* and resulting lesion development. The American Phytopathological Society 73 (3): 494-497. doi: 10. 1094/Phyto-73-494

Tamietti, G. and A. Matta. 2003. First report of leaf blotch caused by *Marssonina coronaria* on apple in Italy. Plant Disease 87 (8): 1005-1005. doi: http://dx.doi.org/10.1094/PDIS .2003.87.8.1005B

Tuite, J. 1969. Plant pathological methods: Fungi and bacteria. Minneapolis: Burgess Publishing Company. p. 239.

Zhao, H., L. Huang, C.L. Xiao, J. Liu, J. Wei and X. Gao. 2010. Influence of culture media and environmental factors on mycelial growth and conidial production of *Diplocarpon mali*. Letters in Applied Microbiology 50 (6): 639-544. doi: 10.1111/j. 1472-765X. 20 10.02847.x